Lack of association of *CR1*, *PICALM* and *CLU* gene polymorphisms with Alzheimer disease in a Polish population

Wpływ wybranych polimorfizmów genów: CR1, PICALM i CLU na chorobę Alzheimera w populacji polskiej

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Abstract

Background and purpose: Recent genome-wide association studies have indicated 3 new susceptibility loci for Alzheimer disease (AD): complement receptor 1 (CR1), clusterin (CLU), and the phosphatidylinositol-binding clathrin assembly protein (PICALM). We investigated the influence of the rs6656401 single nucleotide polymorphisms (SNP) of the *CR1* gene, the rs3851179 SNP of the *PICALM* gene, and the rs11136000 SNP of the *CLU* gene on risk of AD in a Polish population.

Material and methods: In 253 Caucasian AD patients and 240 controls, analyses identifying the rs6656401, rs3851179 and rs11136000 SNPs and *APOE* common polymorphisms were performed.

Results: No significant differences were observed in the distribution of the rs6656401 of *CR1*, rs3851179 of *PICALM* and rs11136000 of *CLU* SNPs between AD patients and controls. The *APOE ε4* common polymorphism was strongly related to the risk of AD.

Conclusion: Our results suggest that investigated SNPs are not associated with AD in a Polish population.

Key words: Alzheimer disease, *CR1*, *PICALM*, *CLU*, single nucleotide polymorphism.

Streszczenie

Wstęp i cel pracy: Metodą przeszukiwania genomu ludzkiego (*genome-wide associations study* – GWAS) stwierdzono związek pomiędzy sporadyczną postacią choroby Alzheimera a genem kodującym główny receptor dla składowej C3b dopełniacza (CR1), intronem genu dla klasteryny (CLU) oraz genem białka błon synaptycznych (PICALM). Celem obecnego badania była ocena wpływu wybranych polimorfizmów: rs6656401 genu *CR1*, rs3851179 genu *PICALM* oraz rs11136000 genu *CLU* na ryzyko rozwoju choroby Alzheimera w polskiej populacji.

Materiał i metody: W badaniu oznaczono polimorfizm rs6656401 genu *CR1*, rs3851179 genu *PICALM* oraz rs11136000 genu *CLU*, a także apolipoproteiny E (*APOE*) u 253 osób ze sporadyczną postacią choroby Alzheimera i 240 osób z grupy kontrolnej.

Wyniki: Rozkład wybranych polimorfizmów u osób z chorobą Alzheimera i w grupie kontrolnej był podobny. Badanie potwierdziło, że *APOE ε4* jest czynnikiem ryzyka rozwoju choroby Alzheimera.

Wniosek: Polimorfizmy rs6656401, rs3851179, rs11136000 nie są czynnikami ryzyka wystąpienia choroby Alzheimera o późnym początku w populacji polskiej.

Słowa kluczowe: choroba Alzheimera, *CR1*, *PICALM*, *CLU*, polimorfizm pojedynczego nukleotydu.

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Introduction

Mutations in the amyloid precursor proteins presenilin-1 and -2 account for most cases of early onset Alzheimer disease (AD) but only for 1-5% of all AD cases. The late onset form of the disease (LOAD) is poorly defined genetically, and until recently the only known risk factor was the ε4 allele of *APOE*. Because it is estimated that *APOE* may account for up to 50% of AD genetic susceptibility [1], other genetic factors must play a role in pathogenesis or protective mechanisms of AD.

So far, more than six hundred genes have been investigated as susceptibility factors for LOAD. Recently published genome-wide associations studies identified three new potential loci for AD: complement receptor 1 (*CR1*), clusterin (*CLU*), and the phosphatidylinositol-binding clathrin assembly protein (*PICALM*).

The protein products of these genes might influence β-amyloid clearance and thus influence the accumulation of amyloid pathology by preventing amyloid plaque formation [2,3].

In the present study we investigated the *CR1* rs6656401, *CLU* rs3851179 and *PICALM* rs11136000 single nucleotide polymorphisms (SNPs) and *APOE* common polymorphisms that were shown previously to affect the risk of AD in different populations in a sample of Polish patients with LOAD and compared them with a group of non-demented controls.

Material and methods

Subjects

The consecutive patients, without a family history of AD, were recruited from the Outpatient Memory Clinic, Neurology Department, University Hospital in Krakow. The clinical diagnosis of probable AD was made according to the National Institute for Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria [4]. Onset of AD was defined as the age at which memory loss or change in behavior was first noted. The evaluation included medical, neurological and neuropsychological examination, interview with a close informant, laboratory testing and computed tomography or magnetic resonance imaging. The details of patient's evaluation are described elsewhere [5].

The patients from the control group were consecutively recruited from the population of Krakow. They had no apparent neurological or psychiatric disease, cerebrovascular disease, or any functional limitations and obtained > 26 points on the Mini-Mental State Examination (MMSE) [6]. Controls and AD patients were Caucasian and of Eastern European descent.

All participants gave informed consent and the study was approved by the Local Ethical Committee.

DNA analyses

The *CR1* rs6656401, *CLU* rs3851179 and *PICALM* rs11136000 SNPs and the *APOE* common polymorphism were studied. DNA was extracted from leukocytes using the standard protocol. The individual genotypes for rs6656401, rs3851179 and rs11136000 SNPs were determined using the polymerase chain reaction restriction fragment length polymorphism method. Real-time PCR was performed using a protocol provided by Applied Biosystems to assess *CR1* rs6656401, *CLU* rs3851179 and *PICALM* rs11136000 polymorphisms.

To determine the *APOE* genotype (*APOE ε2, APOE ε3, APOE ε4* alleles), we genotyped two single-nucleotide polymorphisms (SNPs; NCBI SNPs rs429358 and rs7412) using TaqMan assays (Applied Biosystems [ABI], Foster City, CA).

Statistical analyses

Demographic data between groups were compared by χ^2 (gender) or *t*-test (age).

The genotype and allele frequencies of the *CR1*, *CLU*, *PICALM* SNPs were compared between cases and controls using the χ^2 test (SAS Genetics 9.1). For non-normally distributed variables differences among the groups were tested by Mann-Whitney *U*-test. The Hardy-Weinberg equilibrium was verified for all polymorphisms in the tested population. The level of significance was set at $p < 0.05$.

Results

We studied a total sample of 493 subjects. The AD group consisted of 253 patients (mean age: 73.9 ± 5.2 years, 173 females [68.4%]). The control group included 240 unrelated caregivers or volunteers (mean age: 73.8 ± 6.9 years; 138 females [57.5%]). There was no difference in age between the two groups. The groups differed significantly regarding gender ($p = 0.01$). *APOE ε4* was strongly related to the risk of AD (OR = 6.17; CI: 4.01-9.49).

We did not find a significant difference of three studied polymorphisms between the populations. Table 1 shows the details.

Table 1. Genotype distribution of three studied SNPs in patients with Alzheimer disease (AD) and controls

	AD patients N = 253	Controls N=240	P-value
rs665640			
A/A	22	13	0.35
G/G	124	125	
A/G	107	102	
rs6656401			
C/C	77	92	0.09
T/T	38	40	
C/T	138	108	
rs3851179			
C/C	100	99	0.27
T/T	24	34	
C/T	128	110	

Discussion

We studied 3 different SNPs of genes which encode proteins potentially involved in pathophysiology of AD [7]. Clusterin, also called apolipoprotein J, is a protein involved in membrane recycling and apoptosis. Clusterin binds in a specific and reversible manner with soluble β-amyloid and forms complexes that cross the blood-brain barrier [8]. Clusterin was also found in amyloid plaques [9]. The gene encoding clusterin, *CLU* (chromosome 8), was investigated as a susceptibility factor for LOAD in genome-wide association studies and candidate gene studies. Meta-analytic data showed that clusterin rs11136000 SNP is associated with LOAD in Caucasian subjects [7].

Phosphatidylinositol-binding clathrin assembly protein is a key component of clathrin-mediated endocytosis. AD brains show a reduced number of synapses. PICALM is involved in a process that is crucial to the functional integrity of synapses. PICALM could promote the synthesis of β-amyloid by regulating endocytosis of endocytic compartments with amyloid precursor protein [10]. The *PICALM* rs 11136000 SNP (chromosome 11) was found to be associated with LOAD in Caucasians [7].

CR1 is a receptor for the complement fragments C3b and C4b and is expressed on many different cell types, particularly in the circulatory system. The complement inhibition was shown to reduce the clearance of β-amyloid in animal models [11]. CR1 is also involved in clearance of peripheral blood β-amyloid from the bloodstream. Meta-analytic data showed the association between *CR1* rs3818361 and LOAD in Caucasians [7].

Although most published studies have found an association between the three studied polymorphisms and AD, we did not find such an association in a Polish population in the present study.

Conclusions

AD is most likely a multifactorial condition, which involves a combination of genetic, lifestyle, and environmental factors. The influence of investigated polymorphisms is probably weak and a very large population is needed to reveal the association. Even though the present study does not confirm the association between the assessed polymorphisms and AD risk in Caucasians, it can be considered valuable as our data can be used for future meta-analyses and help to establish more precisely the role of SNPs in the pathogenesis of AD.

Disclosure

Authors report no conflict of interest.

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